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AUTOMATIC DETERMINATION OF AMMONIA NITROGEN IN MINERAL COMPOUNDS

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[Dosage automatique de l'azote ammoniacal dans les minéralisats]

J. Agneray

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AUTOMATIC DETERMINATION OF AMMONIA NITROGEN IN MINERAL COMPOUNDS

J. Agneray* **

Of all the colorimetric methods proposed for the determination of ammonia nitrogen, the ones which utilize the Berthelot [1] reaction are by far the preferred methods. The great sensitivity of this reaction to contaminations due to atmospheric ammonia is the only relevant criticism that can be made about it. The use of the Technicon analyzer is an effective means of protection against such contamination. We have, therefore, tried to adapt this reaction to the automatic determination of the ammonia nitrogen.

As a starting point for our experiment, we have chosen the work of B. Lubochinsky and J. P. Zalta [2], and P. Fleury and R. Eberhard [3], and have transformed the manual techniques proposed by these authors.

Our main objective was to make the technique suitable for acid mineral compounds, and we selected as the lower limit 30 - 50% (v/V) sulfuric acid.

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Reagents

Reagent 1._ Solution of saturated disodium phosphate.

Dissolve over heat 2 kg disodium phosphate with 12 molecules water in about 6 liters of distilled water. Let this solution stand at room temperature for one week. After the second day, mix from time to time. Filter before use.

Reagent 2._ Diluted sodium hydroxide

Sodium hydroxide solution . . . . . 400 ml,
Distilled water . . . . . as much as is necessary to fill to 2 liters (q.s.p.).

Reagent 3. _ Buffer Solution.

Measure with graduated cylinder:

Saturated solution of disodium phosphate (Reagent 1) . . . . . 4 liters
Diluted sodium hydorxide solution (Reagent 2) . . . . . 1 liter

Shake to mix. Since this reagent has the tendency to form a deposit, it is convenient to prepare it a few days in advance, and to filter it just before use. This reagent is used as diluent in methods A and B, and enters in the composition of all reagents. It is better to avoid the addition of sodium hydroxide to the saturated solution of disodium phosphate in order to avoid the formation of a precipitate of trisodium phosphate, which is difficult to bring again into solution.

Reagent 4._ Phenol solution (Methods A and B)

100% phenol . . . . . 50 g,
Buffer solution (Reagent 3) . . . 1 liter,
Distilled water . . . . . as much as necessary to fill to 2 liters (q.s.p.).

Reagent 5._ 4% chlorometric solution of sodium hypochlorite (Method A)

Concentrated solution of hypochlorite (titrated by iodometry) . . . . . q.v.,
Buffer solution (Reagent 3) . . . . . 1 liter,
Distilled water . . . . . as much as is necessary to fill to 2 liters (q.s.p.).

Reagent 6._ 1.5% chlorometric solution of sodium hypochlorite (Method B)

Concentrated solution of sodium hypochlorite . . . q.v.,
Buffer solution (Reagent 3). . . . . 1 liter
Distilled water . . . . . as much as is needed to fill to 2 liters (q.s.p.).

N.B. — The bleaching solutions (28 - 30%) are relatively stable. It is sufficient to titrate every 1 liter lot. Over a three month period there is only a small percentage variation in the titer value, and this does not cause any inconvenience.

Reagent 7._ Stock solution of sodium nitroprussiate

One percent solution in distilled water. If protected from direct light, it can be preserved without difficulty.

Reagent 8._ Diluted solution of sodium nitroprussiate at 1/2000 (Method A)

Stock solution of nitroprussiate(Reagent 7) . . . 100 ml,
Buffer solution (Reagent 3) . . . . . 1000 ml,
Distilled water . . . . . as much as necessary to fill to 1000 ml (q.s.p.).

Reagent 9._ Diluted solution of sodium nitroprussiate (1/10,000) (Method B)

Stock solution of nitroprussiate(Reagent 7) . . . 20 ml,
Buffer solution (Reagent 3) . . . . . 1000 ml,
Distilled water . . . . . as much as necessary for 2000 ml (q.s.p.).

Reagent 10._ Acid solution

Distilled water . . . . . 800 ml,
Sulfuric acid (Merck or R.P.*). . . 80 ml,
Allow to cool.
Distilled water . . . . . as much as necessary for 1 liter (q.s.p.).

This reagent must be kept inside a stoppered flask. It must not be kept open for more than a few seconds. Dilute this reagent to 1/2 with distilled water (Reagent 10 b) in order to obtain 100% transmission.

Reagent 11._ Stock solution of ammonium sulfate (1 mg nitrogen per ml)

Dry ammonium sulfate (R.P.) . . . . . 4.714 g,
Distilled water . . . . . as much as necessary to fill to 1 liter (q.s.p.).

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*Translator's note: Rhone Poulenc.

This keeps well in a stoppered flask. We have always obtained good results with R.P. ammonium sulphate. It is convenient to check the titer in ammonia nitrogen for each individual lot.

Reagent 12. Standard solution of ammonia nitrogen

Stock solution q.v.,
Diluted sulfuric acid (Reagent 10) . . . 100 ml,
Distilled water . . . . . as much as is necessary for 200 ml (q.s.p.).

This can be preserved well in a polyethylene flask.

Observations. — All reagents are prepared with the use of the graduated cylinder. When starting a series of automatic determinations, the reagent bottles must be full so that the reagent is never changed during the determinations. These reagents keep well, although they have a tendency to form a deposit similar to that of the buffer solution (Reagent 3). They should be filtered before use. We prefer to prepare the reagents every day and this, as also for the stock solutions, does not require but a few minutes.

Suggested Methods

Method A. With dialysis

Figure 1 shows the outline of the cycle for the determination of ammonia nitrogen by dialysis. Figure 2 gives a calibration curve, and Figure 3 — an example of recording. It is possible to make between 40 and 60 measurements per hour. In any case, the recording is excellent. This technique can be used for concentrations between 2.5 and 90 µg/ml of ammonia nitrogen. It is easy to modify the cycle in order to adapt the method to higher concentrations without previous dilution of the mineral compounds. It is enough to reduce the diameter of the sampling tube, and to use absorption cells of 3 mm.

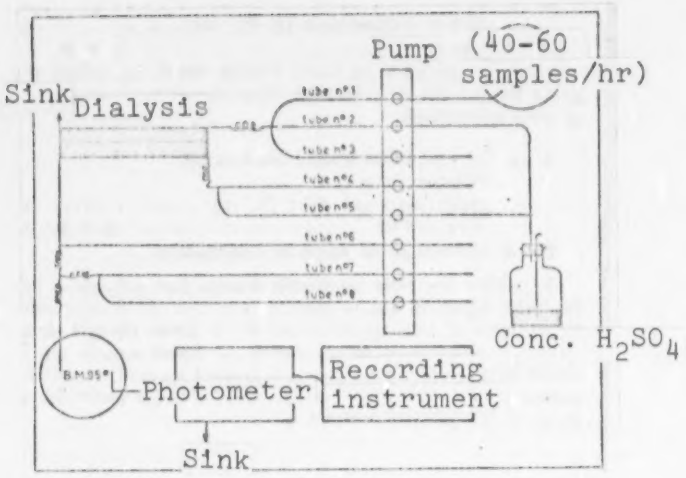


Figure 1. Outline of cycle for determination of ammonia nitrogen by means of a Technicon auto-analyzer:

Method A: with dialysis

Tube no. 1: ϕ 0.056" — yellow — sampling;
Tube no. 2: ϕ 0.065" — blue — air;
Tube no. 3: ϕ 0.081" — purple — buffer solution (Reag. 3);
Tube no. 4: ϕ 0.081" — purple — buffer solution (Reag. 3);
Tube no. 5: ϕ 0.056" — yellow — air;
Tube no. 6: ϕ 0.065" — blue — phenol solution (Reag. 4);
Tube no. 7: ϕ 0.045" — red — sodium hypochloriate solution (Reag. 5);
Tube no. 8: ϕ 0.030" — black — sodium nitroprusside solution (Reag. 8);
photometer — absorption cells of 6 mm; filter — 625 mμ;
recording instrument — 100% transmission is obtained by drawing up sulfuric acid diluted to 40% (Reagent 2) using the sampling tube

Method B. Without dialysis

Figure 4 shows the setup for dosage, without dialysis, of ammonia nitrogen in a mineral compound. Figure 1 gives an example of a calibration curve, and Figure 5 — the corresponding recording. This technique is used for levels between 30 and 50 μg/ml ammonia nitrogen. Levels between 1 and 30 μg/ml can be measured with a little less accuracy. It is very easy to adapt this technique to higher concentrations: to accomplish this, it is sufficient to increase the diameter of the sampling tube or to reduce the volume of the diluents. It is necessary, in this case, to do some preliminary

testing in order to make sure that the buffering action is sufficient to neutralize the relatively higher concentration of sulfuric acid.

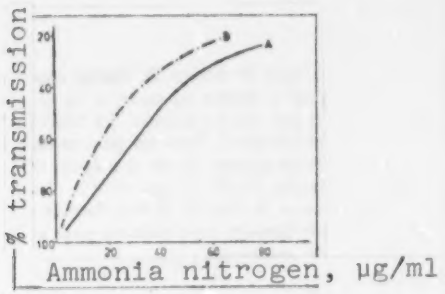


Figure 2. Calibration curves for ammonia nitrogen from values obtained with the autoanalyzer Technicon:

Curve A (——) — Method A, outline from scheme in Figure 1; curve B (---) — Method B, outline from scheme in figure 2

Discussion

The refinement of the technique that we propose was reached after a large number of experiments. For the discussion that we are about to present, we have analyzed 150 readings of gamma standards under different conditions. On the other hand, our study deals with approximately 2000 determinations of ammonia nitrogen which were carried out on mineral derivatives of protein phytate.

We will decide later which experimental data seem to be of importance in order to obtain accurate determinations.

1. Selection of the sensitivity of the method

The technique of B. Lubochinsky and J. P. Zalta is a highly sensitive one. It is possible with a Technicon autoanalyzer to determine 0.1 µg/ml with an accuracy of 0.01 µg/ml. We purposely tried to avoid this sensitivity. In practice, with respect to the problem in which we are interested, the nitrogen content of the sample is not a limiting factor, at least within the sensitivity limits of this technique.

Our measurement of the number of basic groups of serum proteins can be carried out on a 1 ml sample with a 15% dilution of the serum under study. After precipitation, mineralization and bringing to a 25 ml volume, the level of ammonia nitrogen is 25 - 30 µg/ml. If we further dilute our mineral compound (by reaching, for instance, a volume of 125 ml) in order to obtain levels of 5 - 6 µg/ml, we are increasing the errors due to contaminations caused by the nitrogen of the distilled water (0.25 µg/ml).

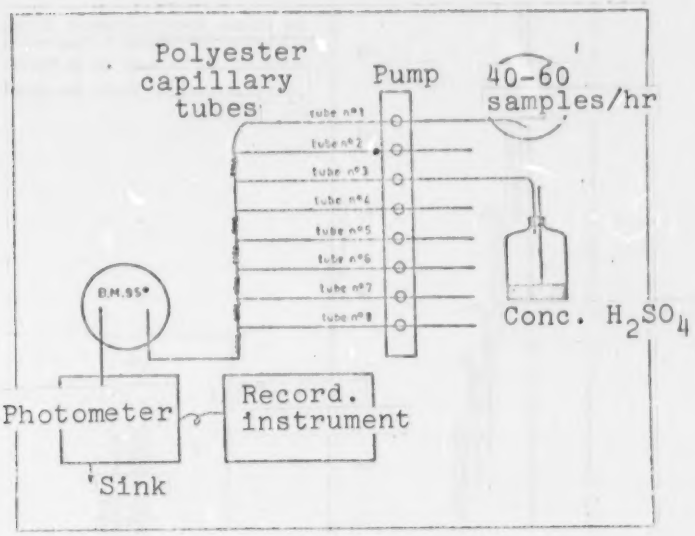


Figure 3. Outline of cycle for determination of ammonia nitrogen by means of autoanalyzer Technicon:

Method B: without dialysis

Tube no. 1: ϕ 0.030" — black — sampling;
Tubes nos. 2, 4, 5: ϕ 0.081" — purple — buffer solution (Reag. 3);
Tube no. 3: ϕ 0.090" — purple & black — air;
Tube no. 6: ϕ 0.081" — purple — phenol solution (Reag. 4);
Tube no. 7: ϕ 0.081" — purple — sodium hypochlorite solution (Reag. 6);
Tube no. 8: ϕ 0.081" — purple — sodium nitroprussiate solution (Reag. 9);
photometer — absorption cells 6 mm; filter — 625 mμ; recording instrument — 100% transmission obtained by drawing up sulfuric acid diluted to 40% (Reagent 2) with the sampling tube

Note: All T tubes have capillary branches

The error in excess goes from a value of 1% to a value of 5%. For this reason, we have refined a cycle of automatic analysis which allows us to obtain good readings between 10 and 30 μg/ml. In this case, the error due to a good quality distilled water is always lower than 1% and can, in general, be neglected.

Figure 4. Recording curve of a standard gamma of ammonia nitrogen read with the autoanalyzer Technicon:

Method A — outline of Figure 1 (nitrogen levels expressed in µg/ml ammonia nitrogen)

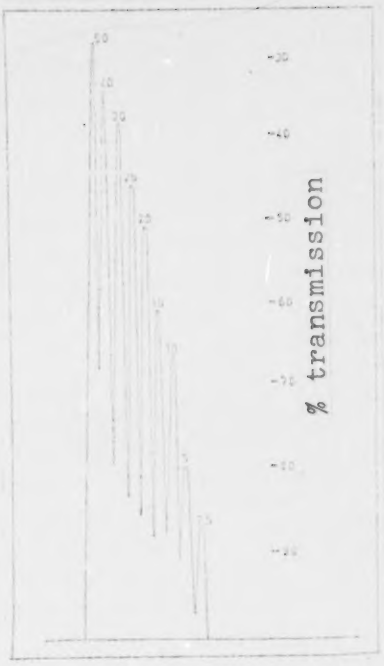


Figure 5. Recording curve of a standard gamma of ammonia nitrogen read with the antoanalyzer Technicon:

Method B — outline of Figure 4 (nitrogen levels expressed in µg/ml ammonia nitrogen)

2. Selection of number of measurements per hour

The preceding considerations entail the selection of a very small sample (black tube: approximate delivery volume 0.32 ml/min) and of a very high dilution (6 purple tubes having a total delivery of 15/ml/min). This refers to Method B. Operating at the rate of 60 samples per minute, during the 20 seconds of interval between sampling, 5 cc reagent will pass inside the absorption cell. This gives an exceptionally good transmission value of 100%. Even by using Method A, where the relative volumes are less likely to give good recording, the curves obtained are still excellent because the dialysis improves the results by 100%.

3. Role of dialysis

The dialysis is, in general, necessary in order to eliminate the large molecules interfering with the results. This does not happen with the mineral compounds. We know that this dialysis is only a partial one. By maintaining constant experimental conditions, the proportion of the dialyzable compounds that can be detected remains constant. This principle of proportionality is at the very base of the mechanism of an autoanalyzer. In the case in which we are interested, we can expect to encounter a first source of error. The speed of dialysis is a function of the pH, and it is necessary to counterbalance the variations of acidity.

In our first experiments we tried to alkalinize the mineral compounds by use of a strong base (sodium hydroxide) with different concentrations. The experimental results are given in Figure 6. It is evident that upon alkalinization of the solution to be dialyzed, there is a significant decrease of the sensitivity. The phenomenon appears very complex. Generally, a high concentration of sodium hydroxide (10 to 20%) increases the permeability of the cellophane membranes used in the dialysis, and this has a tendency to increase the optical densities. Since sodium hydroxide undergoes partial dialysis, it will alkalinize the counter dialysis fluid.

We will see, later on, that sodium hydroxide at low concentration (5%) decreases the intensity of the color. Finally, in the presence of this concentration of sodium hydroxide, ammonia is displaced and the speed of dialysis of the ammonium ion is definitely different than the one of the ammonium gas. Also, cellophane membranes are not very resistant when used over a prolonged period of time in the presence of sodium hydroxide. This involves breakdowns due to the tearing of the membranes. For all these reasons, we gave up the use of a strong alkalinizing agent for our dialysis liquid.

4. Influence of the acidity of the mineral compound

We have conducted our experiments using the two cycles of determination previously described. This allowed us to eliminate the interference due to dialysis by means of one of the two techniques (Method B).

The general principle of our method of study is the following one: two standard series are prepared — one acid, and one neutral (H_2SO_4 40% v/V). These two series will be mainly used during the preliminary studies. Being the first results of interest, we undertake again the study with standard solutions having a constant concentration of ammonia nitrogen and varying acidity (in general, from 0 - 80% v/V H_2SO_4).

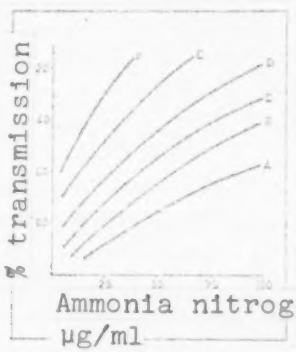


Figure 6. Effect of alkalinization of the samples with sodium hydroxide:

Readings obtained with the Technicon autoanalyzer — cycle shown in Figure 1 with neutral standard sollution.

Dilution of sample with different concentrations of sodium hydroxide (Tube no. 3):

A — 25% sodium hydroxide;
B — 20% sodium hydroxide;
C — 15% sodium hydroxide;
D — 10% sodium hydroxide;
E — 5% sodium hydroxide;
F — distilled water

Counter dialysis (Tube no. 4) is assured with distilled water

a) With dialysis and alkalinization by means of sodium hydroxide

Figure 7 shows that alkalinization with a strong base cannot avoid the error due to the acidity of the sampling. When the diluting liquid contains 15% sodium hydroxide (v/V), the error per excess resulting from measuring the concentration of a mineral compound in relation to a neutral standard scale is 30%. Furthermore, the readings are not exact, due to the fact that the permeability of the dialyzing membrane has a tendency to increase in the presence of sodium hydroxide.

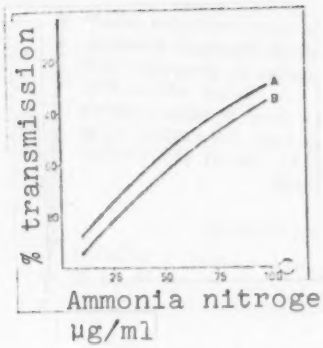


Figure 7. Effect of the acidity of the solution under study on the colored reaction:

Readings made with Technicon autoanalyzer — Method A; cycle of Figure 1; dilution of sample with 15% sodium hydroxide (v/V). Counter dialysis is assured with distilled water

Curve A — acid solutions corresponding to a 40% solution of sulfuric acid (v/V); curve B — neutral solutions

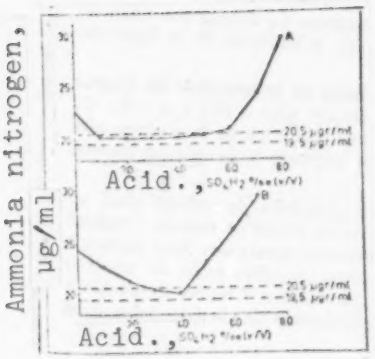


Figure 8. Influence of the acidity of the solution to be measured:

Readings made with Technicon autoanalyzer in relation to the standard scale (Reagent 12)

Curve A — obtained with Method B, cycle of Figure 4; curve B — obtained with more dilute buffer solution: buffer solution, Reagent no. 3, 3 volumes; distilled water, 1 volume

b) Without dialysis and neutralization with disodium phosphate

The best results have been obtained by diluting the mineral compound with a large volume of concentrated disodium phosphate in the presence of an optimum amount of sodium hydroxide.

Figure 8 shows that by using the reagents of the Method B, and an acidity varying between 9 and 60% sulfuric acid, the error of the determination becomes insignificant, and is of the same order as the one which can be made by preparing the scale. It should be pointed out that for buffers with a 25% lower concentration of disodium phosphate, the differences rapidly increase, the farther one goes from the acidity of the standards.

The safety limit, therefore, is not very important, and one should take two precautions. First of all, the mineralization should be accomplished with an exactly measured volume of sulfuric acid. The levels of acid which can be titrated with normal sodium hydroxide do not change, except for a small percentage from one mineral compound to the other (36 $\pm$ 1%, expressed in H_2SO_4 v/V). Secondly, it is to be prefered that work be done in the presence of as high a volume of a buffer solution as possible. It is for this reason that we finally adopted as diluent a solution with 8/10 saturation in disodium phosphate. The addition of 2% sodium hydroxide leads to formation of trisodium phosphate, which is less soluble. It is not, therefore, possible to use a saturated solution. For the preparation of our reagents, we use this buffer diluted to one half, in order to limit the precipitation. For our working conditions this is enough. If one should find it necessary to increase the buffering capacity in order to work with more variable acidities, it would be possible to increase the buffering capacity of the reagent.

c) With dialysis and neutralization with disodium phosphate

Of all the combinations that we have tried with this cycle, Method A gives the best results. The mineral compound is diluted in the buffer (Reagent 3), and this limits the influence of the acidity if the dialysis is speeded. The same buffer (Reagent 3) will act as liquid for counter-dialysis, and will neutralize the sulfuric acid which will pass through the membrane. Using this technique, the standard acid solutions (40% v/V) give optical density values slightly higher than the corresponding neutral solutions. The errors due to slight variations in acidity are insignificant.

5. Study of a sodium borate buffer

We have tried to substitute the solution of disodium phosphate (Reagent 3) with a buffer solution of sodium borate. Using the cycle of scheme no. 4, without the produce of dialysis and the reagents

of Method B, the composition of the diluents in Tubes 3, 4, and 5 is being changed The experimental results can be schematically presented as follows:

a) With a solution of sodium borate close to the saturation value, the optical densities given by the acid standards are definitely weaker than the ones of the corresponding neutral standards. This is due to inhibition of the colored reaction in the presence of acid, and it can very well be attributed to an insufficient buffering capacity of the sodium borate.

b) In media easily buffered with sodium phosphate (with a diluent having the following composition: 2 volumes saturated sodium borate solution, 1 volume saturated disodium phosphate solution), we still encounter a strong difference of optical density between the neutral standards and the ones in acid media. The neutral solutions always give higher optical densities. As shown in Figure 9, there is an unquestionable catalysis phenomenon which is particularly sensitive to a natural medium.

c) In media more strongly buffered by sodium phosphate (with a diluent having the composition: 1 volume saturated sodium borate, 2 volumes saturated disodium phosphate), there is no interference on the part of the sample to be measured. This is due to the fact that the catalysis of the colored reaction is of the same order for both the neutral and the acid standards. In any case, it is less important than in the previous case.

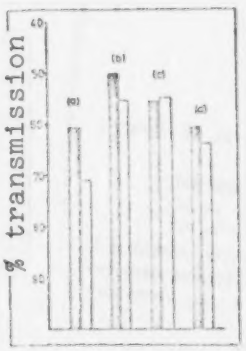


Figure 9. Influence of sodium borate on colored reaction:

Black — neutral solutions; white — acid solutions

d) For comparison, the colors obtained under the same conditions with a colored solution of disodium phosphate are presented in the same graph.

To confirm this interpretation of our results, we prepared standard solutions containing 20 µg/ml of ammonia nitrogen with a 40% v/V acidity due to sulfuric acid, and containing different levels of boric acid (from 0 to 6 g %). In a sufficiently buffered medium (Method B), the optical densities obtained are absolutely independent of the concentration of boric acid. With a buffer solution 25% weaker in disodium phosphate, the recorded optical densities are a function of the boric acid level, as shown the the scheme of Figure 10.

With the cycle shown in Figure 4, good results are obtained when the buffer Reagent 3 is replaced by the following solution:

Saturated solution of sodium borate . . . 200 ml
Pure sodium hydroxide . 30 ml
Saturated solution of disodium phosphate q.s.p. . . . . 1000 ml

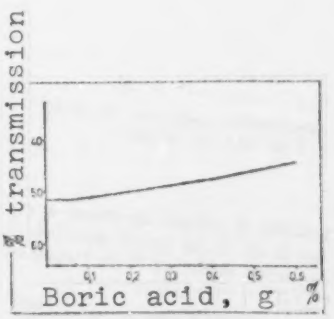


Figure 10. Colors given by 20 µg/ml ammonia nitrogen in the presence of variable amount of boric acid in a strongly buffered medium

However, this reagent does not show any particular advantage in comparison with solution Reagent 3. On the contrary, the risk of interference on the part of the boric ion makes, in certain cases, the technique less exact. This is the reason we finally adopted a buffer solution of sodium phosphate.

6. Effect of alkalinization of media by a small amount of sodium hydroxide

Disodium phosphate is unable to assure an optimum pH for the reaction. It is necessary to add to the medium a certain amount of

sodium hydroxide. In the classical technique, this is assured by the use of the sodium-phenol reagent. We have tried to determine by means of our technique the optimum value for the concentration of sodium hydroxide. We thought it more appropriate to add sodium hydroxide to the buffer Reagent 3 and, therefore, to all reagents, rather than to the sodium-phenol reagent.

In the cycle shown in Figure 4, when disodium phosphate with a saturation of 8/10 (tubes 2, 4, and 5) is used as diluent, the acid samples give results which are definitely too weak in comparison with the neutral standard (curve 10). By adding a percentage of sodium hydroxide, the difference between the acid and neutral samples becomes practically insignificant (Figure 12). With 2% sodium hydroxide, the neutral and acid standard curves are exactly superimposable. Starting from 3%, the optical densities decrease progressively with the alkalinization of the medium, while the acid and neutral solutions give the same readings (Figure 13).

We have obtained very similar results with a buffer having the following composition:

Saturated solution of disodium phosphate . . . 3 volumes
Saturated solution of sodium borate . . . . . 1 volume

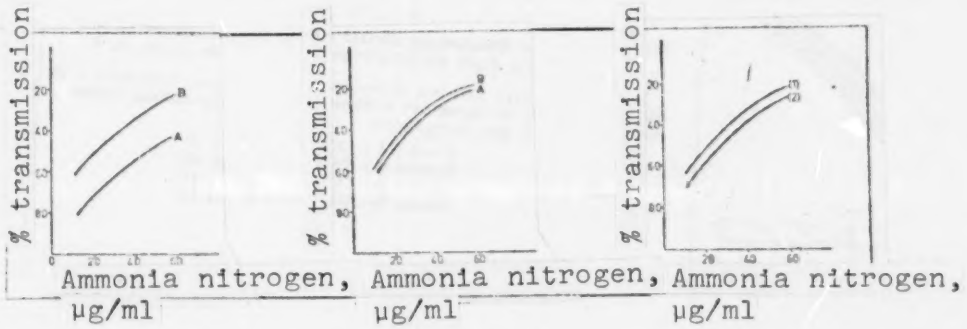
We can conclude from this experience that optimum alkalinization is obtainable by using 2 or 3% sodium hydroxide in all reagents.

7._ Catalysis by sodium nitroprussiate

This is a remarkable catalysis. Without sodium nitroprussiate, a standard solution with 25 µg/ml ammonia nitrogen gives a transmission of 96% which, in practice, cannot be used. The same solution with sodium nitroprussiate gives 45% transmission.

A solution of sodium nitroprussiate(Reagent 9) kept for one week gives exactly the same intensity of color as a freshly prepared

solution. A reagent with double concentration of sodium nitroprussiate(2/10,000) gives an increase in color which is practically insignificant (-1% transmission). A reagent with a concentration five times higher (1,2000) does not cause any additional variation. This reagent can, therefore, be prepared with the greatest freedom.



Figures 11, 12, 13. Influence of the buffer capacity of solution Reagent 3 upon the error due to the acidity of the sample and upon the sensitivity of the reaction:

Figure 11 — Reagent R.3:saturated solution PO_4HNa_2, 800 ml distilled water, 200 ml

Figure 12 — Reagent R.3:saturated solution PO_4HNa_2, 800 ml; distilled water, 190 ml; sodium hydroxide, 10 ml

Figure 13 — Reagent R.3: saturated solution PO_4HNa_2, 800 ml; distilled water, 180 ml; sodium hydroxide, 20 ml

Reagent R.3: saturated solution of PO_4HNa_2, 800 ml; distilled water, 170 ml; sodium hydroxide, 30 ml

Curves A — standard acid solutions (H_2SO_4 40% v/V); curves B — neutral standard solutions; Figure 13 — curves A and B are superimposed

8._ Influence_of_sodium_hypochlorite_concentration

Curves of Figure 4 show that within certain limits the color obtained is an inverse function of the chlorometric degree of the reagents Reagent 5 and Reagent 6. There is, therefore, no advantage in using too concentrated a reagent.

9. Order in which reagents are added.

This order is practically meaningless. The sodium-phenol reagent can be added before buffering the mixture. One can also add all of the reagents, and then dilute with the buffer Reagent 3. Slightly less accurate recordings are obtained when sodium hypochlorite is added before sodium nitroprusside. Contrary to results with the manual technique, the interval of time between the addition of sodium nitroprusside and sodium hypochlorite does not seem to interfere to an appreciable extent. These reagents can be added separately (as in Method B), or mixted together (as in Method A).

10. Effect of catalyzers for mineralization

The copper ions brought in by a catalyzer (Boivin mixture) interfere considerably with the measurements. The color obtained is an inverse function of the content in copper ions. This inhibition is probably connected with the formation of the cuproamine complexes. In order to avoid this error, it is preferable not to use complex-forming cations. As far as we are concerned, we use potassium sulfate in order to increase the temperature, and this does not interfere in any way with colorimetry.

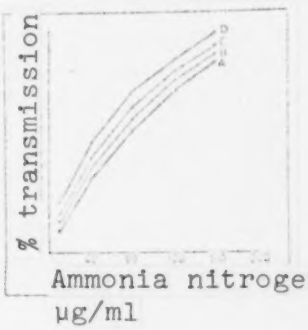


Figure 14. Curves of standardization of ammonia nitrogen as a function of the chlorometric degree of Reagent 5:

Readings with Technicon analyzer: cycle of Figure 1 (neutral standard solutions and non-buffered reagents)

Curve A — ClONa 6°; curve B — ClONa 5°, curve C — ClONa 4°; curve D — ClONa 3°

11. Comments on the setup of cycles

It is especially the scheme of Figure 4 that requires some comment. The sample is very small, and it will have to be diluted slightly with a large volume of reagent.

All reagents are brought into the reaction through the T tubes with capillary branches, and this is necessary in order to obtain a uniform mixture. The main difficulty is to break up the sample with a large number of air bubbles. In effect, if the air volume is too small, the distance between two succeeding bubbles is too great and this gives irregular recordings. It is convenient to dilute the sample before adding air bubbles. This is the only way to obtain a good fractioning of the stream where the reaction is taking place.

Finally, the introduction of the sample inside the diluent is improved when the connection polyethylene tube generally used is replaced with a polyester capillary tube of the same quality as the tube used to take samples from the cups of the sampling tray.

Conclusions

We present two techniques for the automatic measurement of ammonia nitrogen. We have studied these measurement cycles with the purpose of making the measurements directly on the acid liquid of mineralization.

Method A can be used with all media.

Method B is much more accurate when there is a great variation in acidity. It cannot be used in the presence of large molecules which cannot undergo dialysis.

The preparation of the reagents is quick, and it does not require accurate adjustments. Using the precautions we suggest, the errors of measurement are insignificant.

[This is a joint work of the Department of Biological Chemistry at the Faculty of Pharmacy of Paris (Prof. J. E. Courtois) and of the Central Laboratory of Biological Chemistry of the Bichat Hospital Pharmacy (R. G. Douris, Pharmacist of the Hospitals).]

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Following the publication of this manuscript, we became aware of the work published by Assous, E., C. Dreux and M. Girard. Ann. Biol. Clin, Vol. 18, 1960, p. 319.

These authors, for the measurement of very low amounts of ammonia nitrogen in the blood, suggest a technical adaptation different from ours. The comparison between these two techniques shows the importance of the reaction with the phenol-hypochlorite, made more sensitive by sodium nitroprusside, and the extraordinary maneuverability of the Technicon autoanalyzer.

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